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Abstract

The ability of native prairie grasses, big bluestem (*Andropogon gerardii* Vitman), Yellow indiangrass (*Sorghastrum nutans* L.), and switchgrass (*Panicum virgatum* L.), to degrade atrazine and metolachlor was evaluated in two soils denoted as Alpha and Bravo soils. Vegetation significantly decreased the amount of remaining atrazine in Alpha soil when the concentration of atrazine before vegetation was 93 μg g-1, but had no effect on the degradation of atrazine when it was 4.9 μg g-1. The significant effect of the plants on atrazine degradation in Alpha soil occurred at 57 days after the transplanting of vegetation, but not at 28 days after the transplanting of vegetation. The grasses did not enhance the degradation of atrazine in Bravo soil due to the population of atrazine-degrading microorganisms in that soil. The native prairie grasses had a significant positive effect on the enhanced degradation of metolachlor in both soils, and the significant effect was observed at 28 and 57 days after the transplanting of vegetation in Alpha and Bravo soil, respectively. NH4NO3 had no effect on the degradation of atrazine and metolachlor in either soil. Our results indicate that it is feasible to use the native prairie grasses to help remediate the soils contaminated with high concentrations of atrazine and metolachlor, especially in the absence of the indigenous atrazine or metolachlor degraders.

Disciplines

Agricultural Science | Entomology | Other Plant Sciences | Plant Breeding and Genetics

Comments

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Chapter 9

The Use of Native Prairie Grasses to Degrade Atrazine and Metolachlor in Soil

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The ability of native prairie grasses, big bluestem (*Andropogon gerardii* Vitman), Yellow indiangrass (*Sorghastrum nutans* L.), and switchgrass (*Panicum virgatum* L.), to degrade atrazine and metolachlor was evaluated in two soils denoted as Alpha and Bravo soils. Vegetation significantly decreased the amount of remaining atrazine in Alpha soil when the concentration of atrazine before vegetation was 93 $\mu g\ g^{-1}$, but had no effect on the degradation of atrazine when it was 4.9 $\mu g\ g^{-1}$. The significant effect of the plants on atrazine degradation in Alpha soil occurred at 57 days after the transplanting of vegetation, but not at 28 days after the transplanting of vegetation. The grasses did not enhance the degradation of atrazine in Bravo soil due to the population of atrazine-degrading microorganisms in that soil. The native prairie grasses had a significant positive effect on the enhanced degradation of metolachlor in both soils, and the significant effect was observed at 28 and 57 days after the transplanting of vegetation in Alpha and Bravo soil, respectively. NH_4NO_3 had no effect on the degradation of atrazine and metolachlor in either soil. Our results indicate that it is feasible to use the native prairie grasses to help remediate the soils contaminated with high concentrations of atrazine and metolachlor, especially in the absence of the indigenous atrazine or metolachlor degraders.

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Introduction

One problem associated with the widespread use of atrazine (ATR, 2-chloro-4-ethylamino-6-isopropylamino-*s*-triazine) and metolachlor (MET, 2-chloro-N- (2-ethyl-6-methylphenyl)-N- (2-methoxy-1-methylethyl) acetamide) as preemergent herbicides to control grass weeds and broadleaf weeds is the contamination of surface and ground waters (*1-3*). ATR and MET have been frequently detected in rivers and streams of the Midwestern United States (*4-6*). ATR occasionally has exceeded the maximum contamination level for ATR in drinking water (3 $\mu g\ L^{-1}$) set by the U. S. Environmental Protection Agency (*3, 6*). Effective and low-cost remediation approaches at the sites contaminated with ATR and MET are needed.

There is current interest in the use of plants to remediate contaminated soils, sediments, and water, termed phytoremediation. Phytoremediation is an aesthetically pleasing and cost-effective approach for the treatment of hazardous waste sites (*7*). Plants may act directly on organic compounds via uptake of organics and biotransformation of the organics to less toxic metabolites, and/or indirectly degrade them via the rhizosphere effect (*8, 9*). The uptake is influenced by the physicochemical properties of the compounds, the characteristics of the plant species, and environmental conditions (*9-14*). Plants can take up moderately hydrophobic organics (octanol-water partition coefficients, Log K_{ow} = 0.5-3) quite effectively (*12*). More lipophilic compounds (Log K_{ow} > 3.0) can better partition into roots, but they can not easily be transported within the plants. More lipophobic compounds (Log K_{ow} < 0.5) are not sufficiently sorbed to roots or actively transported through plant membranes (*12*). Plant characteristics, such as root surface area, could substantially alter adsorption of an organic compound to roots. A large portion of the applied ^{14}C-ATR (91%) in soil has been shown to be taken up by poplar cuttings (*Populus deltoides nigra* DN34) (*7*). However, only 28, 9.9, and 0.51% of the applied ATR was taken up in corn, *Kochia soparia,* and barley, respectively (*14-16*). A high transpiration rate also can increase the uptake of organic compounds (*9,13,14*). Uptake by plants also depends on the soil concentration of the chemicals and the plant species (*9*). The ATR uptake in sudangrass [*Sorghum sudanense* (Piper) Stapf, var. Piper], grain sorghum [*Sorghum bicolor* (L.) Moench], and corn (*Zea mays* L.) was positively correlated with the concentration in soil (*17*). However, higher uptake for the lower soil concentration of ATR was reported in barley (*16*). The amount of uptake also varies with different soil types used (*7,14*).

The rhizosphere is the region immediately surrounding the roots of a plant. It serves as an enrichment zone for increased microbial activity. Plants not only

release exudates in the rhizosphere for microbial growth or cometabolism, but also harbor microbial consortia and mycorrhizal fungi on root surfaces. A great density and diversity of microorganisms occur in the rhizosphere (*14,18-20*). As a result, enhanced metabolic or cometabolic biotransformation frequently occurs in the rhizosphere. Studies have demonstrated the increased degradation of organics in the rhizosphere of a variety of plant species (*20-24*). However, the rhizosphere effect is short-lived in the absence of plants (*25*), and the relevance of soil-only data to *in situ* plant-microbial interactions is limited. There are also cases in which the rhizosphere has no effect on the mineralization of organic compounds (*14,23*). The objective of this study was to determine the effectiveness of native prairie grasses on the degradation of ATR and MET in two soils.

Materials and Methods

Chemicals. ATR (92.2% pure) and MET (97.3% pure) for treating the soils were obtained from Novartis Crop Protection (Greensboro, NC). ATR (98% pure for analytical standard) was purchased from Chem Service (West Chester, PA).

Soils and Plants. Soils were obtained from two agrochemical dealer sites in Iowa. The two sites, denoted as the Alpha site and Bravo site, are located in northwest Iowa and in central Iowa, respectively. Surface soils (top 15 cm) were randomly collected from the vegetated areas by using hand trowels, and combined for each replication. Three replications of soils were taken, sieved (2.4 mm), and stored in the dark at 4°C until needed. Soils were analyzed by standard methods to determine physical and chemical properties. The Alpha soil had a sandy loam texture with 68% sand, 21% silt, and 11% clay. The organic matter, total nitrogen, pH, and cation exchange capacity were 2.5%, 0.08%, 7.8, and 10.0 meq/100g, respectively. The Bravo soil had a loam texture with 32% sand, 50% silt, and 18% clay. The organic matter, total nitrogen, pH, and cation exchange capacity were 3.9%, 0.22%, 7.5, and 14.1 meq/100g, respectively.

Three species of native prairie grasses, big bluestem (*Andropogon gerardii* Vitman), Yellow indiangrass (*Sorghastrum nutans* L.), and switchgrass (*Panicum virgatum* L.), were utilized in this study. The mixture of grasses was planted in a small tray in the greenhouse until the height range of the grasses was between 10 to 20 cm. Then the root soils of the grasses were washed off with tap water, and the grasses were transplanted into Ray Leach "Cone-Tainers"

(Stuewe & Sons, Inc., Corvallis, Oregon) along with glass wool filling the bottom of each cone and the soils treated with ATR and MET and aged for various days in the following experiments. Each cone contained 6 to 12 grass plants (a mixture of the three species of native prairie grasses).

Alpha Soil Study I. A treating solution consisting of a mixture of ATR and MET was prepared in acetone and applied to Alpha soils at a concentration of 100 μg g^{-1} soil (dry weight) for ATR and 25 μg g^{-1} soil (dry weight) for MET. The chemicals were aged for 13 days in the greenhouse at a temperature of 27 ± 2 °C before the following four treatments were added: addition of NH_4NO_3 (equivalent to 89.7 kg ha^{-1}), vegetation, addition of both NH_4NO_3 (equivalent to 89.7 kg ha^{-1}) and vegetation, and addition of only a phosphate buffer (control). Five mL of water was added per 120-g soil (dry weight) on a weekly basis during the aging period. There were four replications for each treatment, and each replication contained 80 g of soil (dry weight). Analysis by gas chromatography indicated that the soils contained an average of 93 ± 5 μg g^{-1} of ATR and 24 ± 2 μg g^{-1} of MET before the treatment with vegetation and NH_4NO_3. The treated soils were placed in cones, and then the grasses were transplanted in the cones. The cones were kept in the greenhouse, and water was added to the soils on a daily basis to maintain adequate moisture until the end of the study. Concentrations of ATR and MET were determined at 57 days post treatment with vegetation and NH_4NO_3. The reported percentage of remaining ATR or MET at 57 days post vegetation and NH_4NO_3 was calculated by dividing the concentrations of ATR or MET at 57 days post treatment with vegetation and NH_4NO_3 by the concentrations before the treatment with vegetation and NH_4NO_3, then multiplying by 100.

Alpha Soil Study II. Alpha soils were treated uniformly with a mixture of ATR and MET by using acetone as the solvent, providing 100 μg of ATR g^{-1} soil (dry weight) and 25 μg of MET g^{-1} soil (dry weight). After fortification with the chemicals, the soils were placed in cones, and were aged for 50 days in the greenhouse at a temperature of 27 ± 2 °C before the following two treatments were added: vegetation, and control treatment (no vegetation). Five mL of water was added to each cone (103-g soil, dry weight) on a weekly basis during the aging period. There were eight replications for each treatment, and each replication contained 75 g of soil (dry weight). Analysis by gas chromatography indicated that the soils contained an average of 84 ± 23 μg g^{-1} of ATR and 17 ± 3 μg g^{-1} of MET before the treatment with vegetation The aged soils were placed in cones, and then the grasses were transplanted in the cones. The cones were kept in the greenhouse, and water was added to the soils on a daily basis to maintain adequate moisture until the end of the study. Concentrations of ATR and MET were determined at 28 days post vegetation. The reported percentage

of remaining ATR or MET at 28 days post vegetation was calculated by dividing the concentrations of ATR or MET at 28 days post vegetation by the concentrations before vegetation, then multiplying by 100.

Alpha Soil Study III. The Alpha soil used in this study had been fortified with a mixture of ATR and MET (200 μg g^{-1} soil each) and aged for 67 days before remediation, and had been remediated by using the same species of the grasses as in the current study and bacteria for 213 days. At the beginning of the current study, the average concentrations of ATR and MET were 4.9 ± 4.0 and 71 ± 18 μg g^{-1} soil (dry weight), respectively. The soil was divided into two treatment groups. One half of the soil was placed in cones, and the grasses were transplanted in the cones. The other group of cones contained soil only. Each cone contained 90 g soil (wet weight). Each group contained four replicates. The cones were kept in the greenhouse, and water was added to the soils on a daily basis to maintain adequate moisture until the end of the study. Concentrations of ATR and MET were determined at 56 days post vegetation. The reported percentage of remaining ATR or MET at 56 days post vegetation was calculated by dividing the concentrations of ATR or MET at 56 days post vegetation by the concentrations before vegetation, then multiplying by 100.

Bravo Soil Study. The procedures exactly followed the Alpha soil study I except that the Bravo soil was used, and the chemicals were aged for 14 days before the treatment with vegetation and NH_4NO_3. The average concentrations of ATR and MET before the treatment with vegetation and NH_4NO_3 were 69 ± 23 and 31 ± 2 μg g^{-1}, respectively.

Chemical Analysis. After the appropriate time period, the soils were extracted with ethyl acetate three times by mechanical agitation. The extracts were concentrated with a rotary evaporator. The concentrated extracts were analyzed using a Shimadzu GC-9A gas chromatograph (Shimadzu Corporation, Kyoto, Japan) equipped with a flame-thermionic detector. Chromatographic conditions were: injector temperature 250 °C; detector temperature 250 °C; column: glass packed with 80/100 mesh 3% OV-17 (Supleco Inc., Bellefonte, PA), 1.2 m length x 3 mm i.d.; column temperature 230 °C; carrier gas helium; flow rate 45 mL min^{-1}. The extraction efficiency for ATR and MET was 107 ± 9% and 98 ± 0.1%, respectively. The quantitation limit = (the concentration (μg mL^{-1}) of the standards required to give a signal-to-noise ratio of 2:1) * (10 mL of the soil extract)/25 g soil. The quantitation limit for ATR and MET was 0.078, and 0.313 μg g^{-1} soil, respectively. The significance of the differences of the percentage of remaining ATR or MET between vegetated soils and unvegetated soils or between the soils amended with NH_4NO_3 and the soils without NH_4NO_3 was tested by analysis of variance.

Results and Discussion

The mixture of the prairie grasses significantly enhanced the degradation of ATR in the Alpha soil study I (Table I). Our result is consistent with the findings of Arthur et al., who reported that *K. scoparia* significantly decreased the extractable ATR in Alpha soil (*15*). However, the grasses did not significantly enhance the degradation of ATR in the other studies (Table I). The duration of the growth of the grasses in the Alpha soil study I and III was very similar after they were transplanted to the contaminated soils; however, the effect of vegetation on the degradation of ATR was different. The main difference between the study I and III is the different ATR concentrations before vegetation. The average concentration of ATR before vegetation in the study I was much greater than that in the study III. Our results indicate that vegetation can degrade ATR more efficiently when the concentration of ATR is high than when the concentration of ATR is low in soil.

Table I. The influence of a mixture of three native prairie grasses on the degradation of atrazine in Alpha and Bravo soils. Data are reported as percentage of remaining atrazine at the end of each study

	Vegetation	*No Vegetation*	*Pr > F*	*Standard Error of Mean*
Alpha Soil Study I	2.3	21	0.0198	4.92
Alpha Soil Study II	38	42	0.6932	6.45
Alpha Soil Study III	90	85	0.6420	7.01
Bravo Soil Study	14	13	0.4817	0.01

In the Alpha soil study II, the concentration of ATR before vegetation was as high as that in the Alpha soil study I. However, the duration of the growth of the grasses in soils in the study II was only half of that in the study I after they were transplanted to the contaminated soils. The longer duration of the growth of the grasses in the study I probably resulted in the greater uptake of ATR into the plants or greater degradation of ATR in the rhizosphere.

Vegetation had no effect on increasing biodegradation of ATR in Bravo soil. This failure appears to be related to the effective mineralization of ATR by indigenous ATR- degraders in that soil. The number of indigenous ATR-degraders is much higher than that in Alpha soil (*15, 26*). The large indigenous population of ATR-degraders in Bravo soil was effective in mineralizing ATR. Struthers et al. reported that approximately 50% of the applied ^{14}C-ATR (50 μg g^{-1}) was mineralized within 63 days after the treatment of ATR in Bravo soil

(*26*). Perkovich et al. noted that 62 and 49% of the applied ^{14}C-ATR (50 μg g^{-1}) was evolved as $^{14}CO_2$ after 36 days of incubation in *K. scoparia* rhizosphere soil and non-rhizosphere soil from the Bravo site, respectively (*22*).

The addition of the prairie grasses significantly reduced the concentrations of MET in all the studies (Table II). Other researches have showed that corn and aquatic plants, such as coontail (*Ceratophyllum demersum*), American elodea (*Elodea canadensis*), and common duckweed (*Lemna minor*), were effective in enhancing the degradation of MET in soil (*24*) or water (*27*), respectively. In the Alpha soil study II, the grasses significantly decreased the amount of remaining MET, but not ATR. It may be related with the greater water solubility and lipophilicity of MET (530 mg L^{-1} at 20 °C and K_{ow} of 2820, respectively) compared with those of ATR (33 mg L^{-1} at 27 °C and K_{ow} of 219, respectively). The greater water solubility and the lower concentration of MET before vegetation make it possible that a larger percentage of the applied MET is dissolved in the soil water than for ATR. The concentration of the chemical in soil water is a major factor influencing the direct uptake of the chemical through the plant roots (*9*). As a result, a larger percentage of the applied MET may be taken up by the plants compared with that of ATR in the same time frame. In addition, uptake of MET by the plant roots may be greater than that of ATR due to the higher lipophilicity of MET. In a previous study, a significantly greater amount of ^{14}C was found in the plant tissues of the ^{14}C-MET-treated water systems than in the ^{14}C-ATR-treated water systems when both chemicals were applied to water at same concentration (*27*). The lower water solubility and lipophilicity of ATR may explain why more time is needed for the grasses to decrease the remaining ATR significantly in the Alpha soil study II. Another reason for the different vegetation effects on the degradation of ATR and MET in the Alpha soil study II may be that the percentage of MET degraded in the

Table II. The influence of a mixture of three native prairie grasses on the degradation of metolachlor in Alpha and Bravo soils. Data are reported as percentage of remaining metolachlor at the end of each study

	Vegetation	*No Vegetation*	*Pr > F*	*Standard Error of Mean*
Alpha Soil Study I	37	77	0.0001	3.00
Alpha Soil Study II	51	74	0.0013	2.68
Alpha Soil Study III	63	93	0.0394	5.98
Bravo Soil Study	50	67	0.0008	0.03

rhizosphere of the plants is significantly greater than for ATR. Rice et al. indicated that MET was more readily degraded than ATR in water containing live aquatic plants (*27*).

The concentration of MET before vegetation was much greater than that of ATR in the Alpha soil study III. This is probably the reason why vegetation significantly decreased the concentration of MET, but not ATR in that study. The number of indigenous MET-degraders in Bravo soil is very low (*15*). That may explain the different effects of vegetation on the degradation of ATR and MET in the Bravo soil study.

NH_4NO_3 had no effect on the degradation of ATR and MET both in the Alpha soil study I ($P = 0.9404$ for ATR and $P = 0.0734$ for MET) and in the Bravo soil study ($P = 0.8049$ for ATR and $P = 0.7461$ for MET). In the N-amended soils, the remaining ATR and MET was 11% and 61%, respectively, while 12% of ATR and 52% of MET remained in the nonamended soils in the Alpha soil study I. In the Bravo soil study, the remaining ATR and MET was 13% and 58%, respectively, in the N-amended soils, while 13% of ATR and 59% of MET remained in the nonamended soils. This indicates that NH_4NO_3 did not influence the degradative ability of indigenous ATR or MET degraders in either soil. Our results are consistent with the findings of Entry, who noted that degradation of ATR by indigenous ATR-degraders was not affected by the addition of 400 kg N ha^{-1} in blackwater soils (*28*). However, others reported that exogenous N suppressed the ATR degradation by the indigenous ATR-degraders (*29-31*).

Conclusions

The degradation of ATR by the mixture of native prairie grasses was influenced by the concentration of ATR before vegetation, the presence of indigenous ATR-degraders, and the duration of the growth of the grasses in soil. The grasses significantly decreased the MET residues in both Alpha and Bravo soils. The enhanced degradation shown in our results suggests that phytoremediation with native prairie grasses could provide an inexpensive, effective, and aesthetically pleasing way to remediate soils contaminated with high concentrations of ATR and MET.

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